SPECIFIC PRECIPITIN ANTISERUMS FOR KIDNEY PROTEIN

BY

BESSIE GENEVIEVE OSGOOD

A.B., Indiana State College, 1932
B.S., Indiana Central College, 1933
M.S., University of Illinois, 1940

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY IN BIOLOGICAL CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1947

CHICAGO, ILLINOIS
1951

SPECIFIC PRECIPITIN ANTISERUMS FOR KIDNEY PROTEIN

BY

BESSIE GENEVIEVE OSGOOD

A.B., Indiana State College, 1932
B.S., Indiana Central College, 1933
M.S., University of Illinois, 1940

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY IN BIOLOGICAL CHEMISTRY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1947

CHICAGO, ILLINOIS
1951

ACKNOWLEDGMENT

The author wishes to express her appreciation of the advice and assistance given her by Dr. William H. Welker and Dr. George E. Wakerlin in the direction of this investigation. She is also indebted to Dr. Ann Freeman for her cooperation in performing autopsies and to Dr. C. A. Krakower for his kindness in making the histological examinations.

R. UNIV. BIBLIOTHEEK LEIDEN

Since the first preparation of precipitin antiserums for kidney protein by Lindemann in 1900 many studies have been made of the nephrotoxic effect of such antiserums. In the earlier work the injection of an antiserum for homologous kidney into a normal animal was followed by generalized toxic effects which masked the rather feeble nephrotoxic action of the kidney precipitin. Most investigators regarded the nonspecific effects as being due largely to the hemolysins and hemagglutinins and in a smaller measure to precipitating antibodies to non-organ tissues which were present in the anti-kidney serums. Later investigators using improved technics for the preparation of anti-kidney serums were able to produce kidney precipitins relatively but not absolutely free of these interfering antibodies.

The present investigation was undertaken to study further the method introduced by Buchholz for the preparation of anti-dog kidney serums free of precipitin to the dog serum proteins, and to study the nephrotoxic effect of these relatively pure anti-kidney serums.

The material used for production of anti-kidney serum consisted of dog kidneys which were first ground in a food chopper, washed with 0.9 per cent saline solution to remove most of the blood, ground for 48 hours in a ball mill with an equal volume of 0.9 per cent saline and a little toluene, and finally washed repeatedly with saline until the supernatant fluid after centrifugation gave no Biuret test for protein. The finely ground kidney tissue was made into an approximately 1 per cent suspension with saline, fixed on aluminum hydroxide cream and injected intramuscularly into rabbits, each rabbit receiving 20 cc. of the preparation.

Precipitins for dog kidney appeared in the blood of the rabbit in about twelve days, followed in another two days by precipitins for serum albumin and serum pseudoglobulin. After a period of time varying from one to three months the latter antibodies disappeared from the blood of the rabbits leaving a relatively pure anti-dog kidney serum (which is called "pure" for the sake of brevity in the pages to follow although the term is admittedly inaccurate).

Albumin and pseudoglobulin were prepared from defibrinated dog blood by fractional precipitation with ammonium sulfate, following Briggs' modification of Welker's method. Antiserums to each of these proteins were prepared by intramuscular injection into rabbits of a preparation of each protein fixed on aluminum hydroxide cream. Precipitating antibodies to the protein appeared in the blood of the rabbit in high titer in two or three weeks.

Precipitin titers were determined by the contact method. Appropriate dilutions of the antigen were made and a few drops of each dilution were

layered over a few drops of the undiluted specific antiserum. The highest dilution of antigen was determined which gave a white layer of precipitate at the interface after one hour at room temperature. Titers of 50,000 and 100,000 were satisfactory for the serum protein precipitins.

Antigen for testing kidney precipitin titers was prepared by the autolysis for 72 to 80 hours of ground kidney tissue which had been previously frozen and thawed on three separate occasions. "Pure" anti-kidney serums with a titer of 80 or 100 were considered potent and an anti-kidney serum with a titer of 40 was fairly satisfactory.

Investigation of the nephrotoxic action of anti-dog kidney serums prepared by this method involved thirty-four experiments performed on twenty-six dogs, and included studies on the potency and dosage of anti-dog kidney serum required to produce a definite nephrotoxic action and the possible effects of anesthesia, route of injection and the presence of serum protein antibodies on the response of the kidney to the antiserum.

The experiments fell into three general groups. The eighteen dogs in Group A received pure anti-dog kidney serums of varying potencies and in different dosages. Group B consisted of six dogs which were given "mixed" antiserums — that is anti-dog kidney serums plus antiserums to the two serum proteins. The antiserums were either mixed before injection or administered in separate injections about one minute apart. Group C consisted of seven control dogs. One dog was injected with normal rabbit serum, two with anti-sheep kidney serum and four with antiserums to either one or both of the serum proteins. Five dogs received more than one injection, of the same or a different type of antiserum.

The intensity and course of the induced nephritis were studied by means of chemical and clinical tests on the blood and urine of the dogs and in some cases by microscopic examination of kidney sections. The dogs were under observation for periods ranging from six days to one year after the injection.

Twenty-four hour urine samples were collected and examined as to volume, specific gravity, presence of protein and blood, and microscopically for casts and red and white blood cells in the urinary sediment. The identity of the urine proteins was studied by means of the precipitin test, the urines after appropriate dilutions being tested with antiserums for dog serum albumin and pseudoglobulin and kidney protein. The highest dilution of urine was determined which gave a positive reaction with each of the three antiserums.

Blood tests were performed on sixteen of the dogs and included red and white cell counts and determination of concentrations of hemoglobin, nonprotein nitrogen and serum protein, and in addition, in a few cases the carbon dioxide capacities and concentrations of albumin, sodium and

chloride of the serum. Blood pressures were studied on fourteen dogs by the method of direct arterial puncture.

Autopsies were performed on six dogs by Dr. G. E. Wakerlin, of the Department of Physiology of the University of Illinois College of Medicine, and on seven dogs by Dr. Ann Freeman, of the University of Illinois. Histological examinations of kidney sections were made by Dr. C. A. Krakower, of the Department of Pathology of the University of Illinois College of Medicine.

The injection of anti-dog kidney serum, either pure or mixed with serum protein precipitins, was followed within twenty-four hours by definite indications of kidney damage in twenty-four of twenty-seven cases. The first indication was always the appearance of protein in the urine. This was frequently but not always accompanied by cylindruria (nineteen cases) and hematuria (sixteen cases). The hematuria was usually very mild, being detected only by examination of the urinary sediment. Changes in urine volume were frequent but not consistent. Sometimes oliguria with an occasional transient anuria was observed but a polyuria of one or two weeks duration was noted just as frequently.

There was a high incidence of lethargy, anorexia, and vomiting during the first two weeks of the induced disease. An occasional case of edema was noted but it was never severe or prolonged. Anemia, nitrogen retention, and hypoproteinemia with lowered albumin-globulin ratios developed in many of the dogs. Significant elevation of the blood pressure however was never found.

Microscopic lesions of the kidneys were present in every case examined during the acute and recovery phases. The lesions were predominantly glomerular and of diffuse nature. Injury to the tubules was either slight or entirely absent.

Immunologic study of the identity of the urine proteins showed that the bulk of the protein was serum albumin and serum pseudoglobulin. Kidney protein was detected in the urine in twenty cases. In the majority of these the amount was just detectable and its presence was transient but in all cases except one its presence in the urine was associated with the presence of casts. The five dogs which excreted large amounts of kidney protein were shown by other tests to have a severe grade of nephritis. On the other hand several dogs which were acutely ill of the induced nephritis showed either no kidney protein at all or only a trace during the entire course of the disease, although in four of these dogs it was detected in the blood stream. Although the correlation between the degree of kidney damage and that of kidney proteinuria is thus seen to be incomplete it should be emphasized that kidney protein was never detected in the absence of other signs of kidney damage.

The results of injection of "mixed" antiserums were comparable to those obtained when pure anti-dog kidney serum of the same potency was injected in equivalent amounts. No reactions were ever noted which could not be attributed to the nephrotoxic factor of the serums. The administration of serum protein precipitins alone had no nephrotoxic effect. The results of injection of normal rabbit serum or anti-sheep kidney serum were also negative.

A dose of 2 cc. per kilogram of pure anti-dog kidney serum with a potency of 80 or 100 invariably produced a severe grade of antiserum nephritis. With less potent antiserums or smaller doses the nephrotoxic action while definite and specific was not as consistent or as powerful.

Of four dogs which were under ether anesthesia at the time of injection not one showed any alteration of response to the anti-kidney serum which could be attributed to the effect of the anesthetic. The same was true of five dogs which were under sodium pentobarbital anesthesia at the time of the injections which were made directly into the renal arteries. Injection into the renal artery produced results comparable to those obtained when the saphenous or femoral vein was used.

The dogs in the present series tended toward the extremes of complete recovery or death in the acute stage. Four of the dogs died, in 1, 7, 9 and 13 days respectively after the injection, the last three in uremia. Of the remaining twenty dogs which developed nephritis only three had failed to show a fairly complete recovery at the end of three and a half months.

CONCLUSIONS

1. A potent anti-dog kidney serum has been prepared by a single intramuscular injection into rabbits of well-washed ground dog kidney tissue fixed on aluminum hydroxide cream. One to three months after the injection precipitins to the dog serum proteins have disappeared and a pure potent anti-dog kidney serum has been obtained.

2. The injection of pure anti-dog kidney serum into dogs was followed by the development of acute antiserum nephritis, the severity of which was dependent in large measure on the potency and dose of the antiserum.

3. The administration of antiserums to the two dog serum proteins along with that of anti-dog kidney serum had no clearly defined influence on the severity and course of the disease.

4. The injection of normal rabbit serum and of rabbit serum containing precipitins to sheep kidney or to dog serum proteins had no effect on the kidneys of the recipient dog.

5. The presence of kidney protein in the urine as determined by the precipitin test occurred only in cases of detectable kidney damage.

VITA

Bessie Genevieve Osgood was born in Brook, Indiana, October 2, 1904. Received elementary education in Milford, Illinois. Entered Indiana Central College in 1922 for two years' work and returned in 1930. Received A.B. degree summa cum laude in 1932, B.S. degree in 1933 from Indiana Central College, M.S. degree in 1940 from University of Illinois.

Laboratory and research assistant in Department of Physiological Chemistry of the Chicago Medical School from 1935 to 1944, the last year of which she was an instructor. Research assistant in biochemistry at Hektoen Institute for Medical Research of Cook County Hospital the last half of 1944 and all of 1945. Research assistant, Department of Biological Chemistry of the University of Illinois College of Medicine, 1946 and first half of 1947. Associate member of Sigma Xi, Chicago Colleges of the University of Illinois Chapter.

LIST OF PUBLICATIONS

A Photoelectric Method for the Microdetermination of Sodium in Serum and Urine by the Uranyl Zinc Acetate Precipitation (with W. S. Hoffman). J. Biol. Chem. 124:347, 1938.

The Photoelectric Microdetermination of Nitrogenous Constituents of Blood and Urine by Direct Nesslerization (with W. S. Hoffman). J. Lab. Clin. Med. 25:856, 1940.

A Microphotoelectric Method for Urea Determination in Blood and Urine by Direct Nesslerization (with W. S. Hoffman). J. Lab. Clin. Med. 25:862, 1940.

The Value of Blood Chloride and Sodium Determinations in the Diagnosis of Dehydration (with W. S. Hoffman). J. Lab. Clin. Med. 27: 1174, 1942.

The Determination of the "Sulfate Index" as a Measure of the Degree of Exposure to Benzene Vapor (with W. S. Hoffman). Amer. J. Clin. Path. 15:293, 1945.

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41549816_0195